Growth of Photoheterotrophic Cells of Peanut (*Arachis hypogaea* L.) in Still Nutrient Medium[1]

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ABSTRACT

Cell suspension cultures were established from the callus proliferation of leaf explants of 10- to 12-day-old seedlings of the peanut (*Arachis hypogaea* L. var. TMV-3). The cells could be cultivated in both agitated and still media, the latter promoting more of chlorophyll (Chl) synthesis. High Chl content (210–240 micrograms Chl per gram fresh weight), yield of free and pipetable cells, presence of all the pigments in the same ratio as that of the leaf tissue, and high rates of O_2 evolution (140–170 micromoles O_2 per milligram Chl per hour) were some of the desirable features of the still-grown cell cultures. However, considerable variations with regard to the above characters were observed between the cell cultures of different varieties of the peanut.

O_2 evolution by the cultured cells was dependent on exogenous supply of HCO_3^-. A well-developed photosynthetic apparatus as evidenced from photosystem I and photosystem II activities of the isolated chloroplasts and variable fluorescence measurements with the cell cultures was further documented by electron microscopic evidence of distinct granal stackings in chloroplasts and sodium dodecyl sulfate-polyacrylamide gel separation of thylakoid membranes into P700 Chl *a* protein complex and light-harvesting Chl *a/b* complex. Evidence is presented for the relative increase in the Chl associated with P700 Chl *a* protein complex in contrast to the light-harvesting Chl *a/b* complex in the cultured cells as compared to intact leaf.

Inasmuch as cell suspension cultures are free of cybernetic complications usually associated with tissue and organ specific activity patterns, they have been the subject of a variety of investigations including photosynthesis. Photosynthetic properties have been studied for cell clone cultures of *Nicotiana tabacum* (2, 5, 18, 24), *Chenopodium rubrum* (12), and *Spinacia oleracea* (8). Despite these investigations, there is still no reliable procedure for obtaining cells with high Chl content capable of good photoautotrophic growth and showing high activities of the component photosynthetic reactions.

There are several factors that may contribute to the inadequacies of most of the existing cell cultures for photosynthetic studies. Based on the results obtained from cell cultures of some C_3, C_4, and CAM plants, we recently suggested that the use of chlorophyllous tissues of leaf and cladophyll as inocula, NAA instead of 2,4-D for callus proliferation, and subsequent growth of the cell cultures in glucose- instead of sucrose-supplemented media in addition to high light intensity right from the start of callus initiation are important in establishing photosynthetically active cell cultures (21). In this paper, we describe methods by which highly dispersed, chlorophyllous, and photosynthetically active cell cultures derived from the callus proliferation of the leaf explants of the peanut (*Arachis hypogaea* L.) a C_3 plant, could be established even in still nutrient medium.

MATERIALS AND METHODS

Plant Materials. Seeds of most of the varieties of peanut (*Arachis hypogaea* L. var. TMV-3) were obtained from Dr. S. N. Nigam, Groundnut Improvement Programme, ICRISAT, Hyderabad and Tamilnadu Agricultural University, Coimbatore, India. Seeds of EC 117010 A NC4-X Virginia peanuts were a gift from Dr. R. O. Hammons, Georgia Coastal Plain Experiment Station, Tifton, GA.

Seeds dusted with 0.1% (w/w) Agrosan-D were germinated in garden soil in earthen pots. The growth conditions were the same as described earlier (21). Ten- to 12-d-old seedlings served as the source of explants used for callus initiation.

Callus Initiation. Young but fully expanded leaves (second or third from above) were excised from the plants; leaflets were dissected out and thoroughly washed in tap water. Surface sterilization of the leaflets consisted of rapid immersion in 70% ethanol followed by submersion during 10 min in 5% (v/v) NaOCl and three rinses in sterile distilled H_2O. Leaf discs of 0.5 cm diameter were punched from the lamina avoiding the midrib and major veins, washed once in sterile distilled H_2O, and transferred to nutrient media in 100-ml Erlenmeyer flasks. The composition of the culture medium was essentially that of Murashige and Skoog (21) supplemented with the following additives: NAA, 2.5 mg/l; BA, 1 mg/l; and D-glucose, 3.5% (w/v). The culture conditions are described elsewhere (21). Highly chlorophyllous clones of cells were selectively isolated from the calli proliferated upon the leaf explants and transferred to fresh agar media. The additives of the subculture medium, however, were: NAA, 0.6 mg/l; BA, 0.2 mg/l; and D-glucose, 2.5%. The stock callus cultures grown in the latter medium for two to three passages of 3 weeks each were used for the initiation of cell suspension cultures.

Suspension Cultures. Stock suspension cultures were initiated by disaggregating the cells of the friable calli, scooping the cell masses out, and reinoculating into liquid medium (125 ml) in 500-ml Erlenmeyer flasks. The growth medium of the suspension culture was identical to that used for subculturing the calli except that the agar was omitted. The culture flasks were sealed with two layers of heavy duty aluminum foil and incubated under still conditions or mounted on a gyrotory shaker at 60 rpm. The cultures were maintained at 28 ± 1°C and illuminated at an irradiance of 300 $\mu E\ m^{-2}\ s^{-1}$ by a bank of white fluorescent tubes (Philips TL 40/33) for a 12-h photoperiod. The irradiance was measured using a UDT-model 40X Opto-Meter. The cells grew rapidly both in still and agitated media. However, growth tended to be in clumps, particularly in the former. To remove the aggre-

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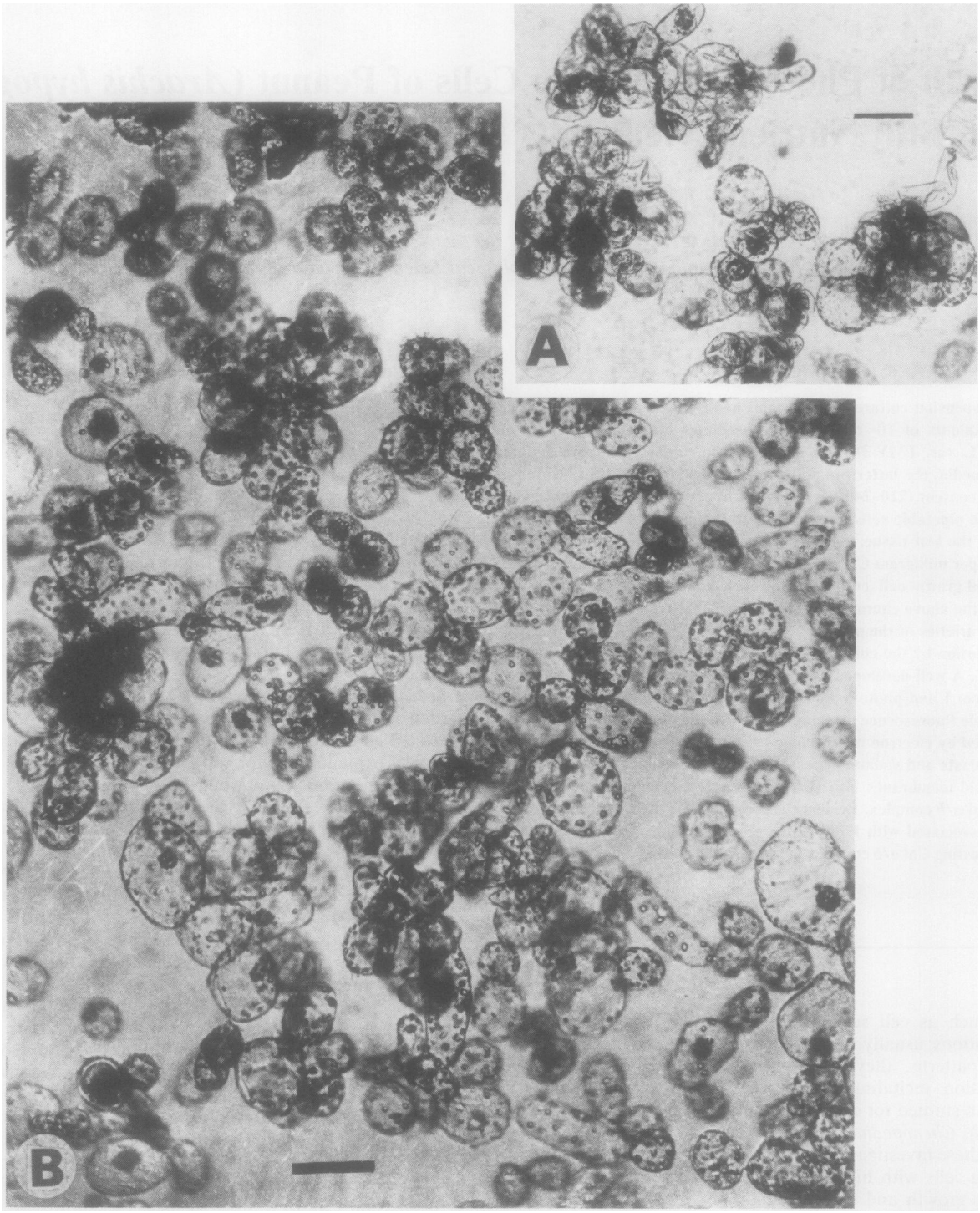

FIG. 1. Light micrographs of still-grown cell suspension of *A. hypogaea* var. TMV-3. A, Cell aggregates in still nutrient medium; B, pipetable free cells and small cell aggregates obtained after mild swirling of the culture flasks. Bar, 100 μm.

gates and to obtain uniform material for inoculation, clumps were disrupted by forcing through a 211-μm nylon sieve fixed to the end of a sterile polypropylene tube (4 cm diameter × 6 cm height). The cells were collected by centrifugation and resuspended in fresh medium. Each new culture was initiated by the transfer of an inoculum containing approximately 10^7 cells into 250-ml flasks charged with 60 ml of the medium. Unless otherwise mentioned, late log-phase (30-d-old) cells were employed for the measurements. All the experimental manipulations were carried out in a sterile laminar air flow system (Atlantis, New Delhi).

Measurements of Growth. Three-ml aliquots of homogeneous suspension were drawn from the culture flasks using a wide mouthed 10 ml pipet and all the following three parameters were employed for measuring the growth of the cells. (a) Changes in PCV.[2] The suspension was pipetted out into locally fabricated hematocrit tubes holding 3 ml and centrifuged at 1000*g* for 2 min.

[2] Abbreviations: PCV, packed cell volume; CPI, P700 Chl *a* protein complex; CPII, light-harvesting Chl *a/b* complex; DCIP, 2,6-dichlorophenol-indophenol; MV, methylviologen.

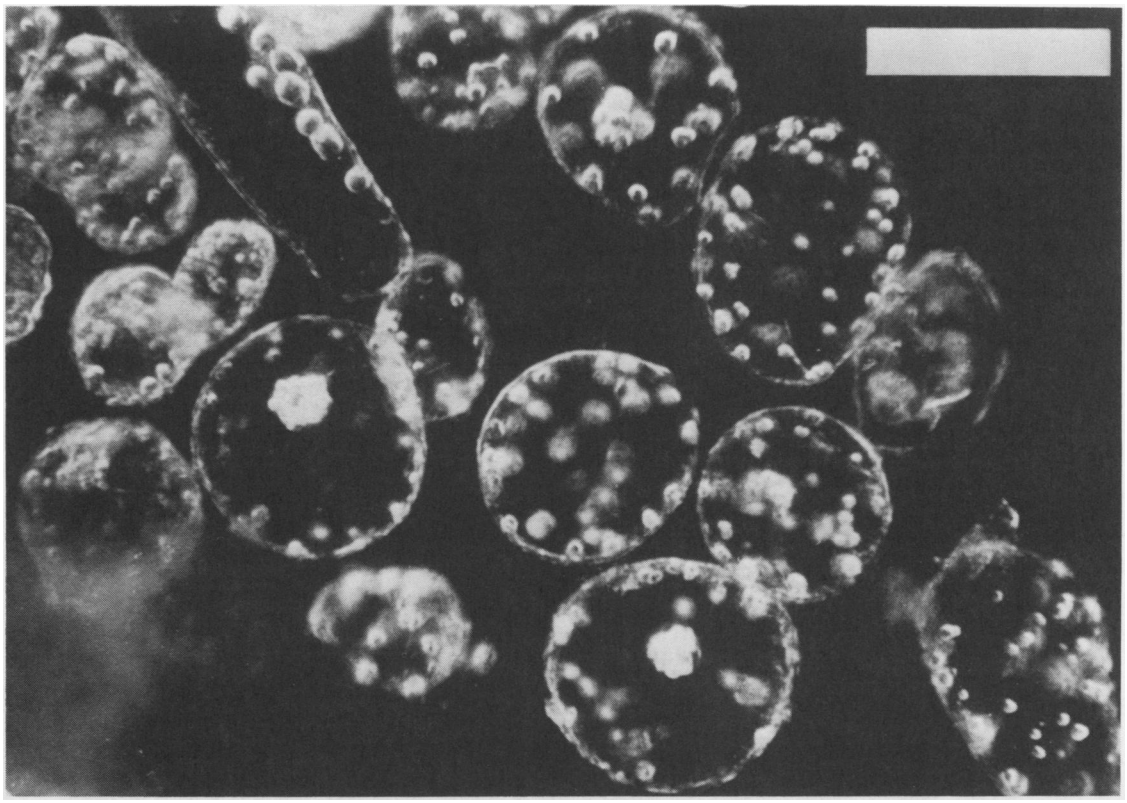

FIG. 2. Light micrograph of still-grown cells of *A. hypogaea* var. TMV-3 in dark field showing distinct chloroplasts. Bar, 100 μm.

Total volume of the cells packed in the graduated region was directly read and the mean values of the replicates were recorded. (b) Changes in cell number were determined by direct microscope counting using a Neubaur improved double Haemocytometer. (c) Changes in dry weight of the cells were determined after drying the sample at 80°C for 24 h (21).

Isolation of Mesophyll Cells. Mesophyll cells were isolated from the leaves by the mechanical grinding procedure of Gnanam and Kulandaivelu (10).

Determination of Free Cell Yield. Two g (fresh weight) of cells and cell aggregates from the still media were transferred to a 211 μm nylon sieve fixed to an end of a polypropylene tube (4 cm diameter × 6 cm height). The end of the tube with the sieve and cells was immersed into 40 ml of liquid nutrient medium in a 100-ml beaker leaving enough space down below for the release of the cells. The cell clusters in the filter were gently stirred with a Teflon rod. After 2 min, the cells released into the medium were collected by centrifugation. The fresh weights of the cells still remaining on the filter and collected by centrifugation were determined. The percent yield of free cells was calculated by assuming that the total release of 2 g of cells into the medium accounted for 100%.

Separation of Pigments by TLC. Cultured cells and leaf tissue (1 g each) were homogenized in 5 ml of 90% methanol to extract the pigments. The residual colorless remains of the cells/tissue were removed by centrifugation, and the volume of the supernatant was reduced to 1 ml under reduced pressure in dark at 25°C. The pigments in the extract (0.2 ml) were separated by TLC using cellulose (TLC grade; BDH, India) and the solvent system consisting of methanol, acetone, and water (15:5:1) and identified according to the method of Randerath (19). Elution of the pigments from the chromatogram and estimation of the individual pigments were done using the solvents and extinction coefficients described by Hager and Meyer-Bertenrath (11).

Isolation of Chloroplasts. Chloroplasts from the precooled cell cultures were isolated by the method described previously (21). Inasmuch as the peanut plants yielded more intact cells than plastids by usual homogenization procedures, acid-washed sand was added at the grinding stage to isolate chloroplasts from the leaves. The homogenate was filtered through four layers of cheese cloth, the filtrate was centrifuged at 500*g* for 1 min to remove the sand particles and broken cells, and the supernatant was further centrifuged at 3000*g* for 3 min. The pelletted chloroplasts were washed once in the same medium.

Microscopy. Light micrographs were taken of the still-grown cells and chloroplasts with a Carl Zeiss photomicroscope.

For electron microscopy, cells were fixed with 2% glutaraldehyde in 100 mM Na-phosphate (pH 7.2) at 4°C for 4 h. Samples were washed with phosphate buffer, postfixed with 2% OsO_4 in buffer for 2 h, dehydrated with a graded acetone series, and embedded in Epon-araldite mixture. Thin sections taken on a LKB Ultratome II were stained with 2% aqueous uranylacetate for 1 h, followed by 10 min for Reynold's lead citrate. Observations and photographs were made on a Siemans Elmiskop I.

Polyacrylamide Gel Electrophoresis. Chloroplasts from the cell cultures and leaf cells were isolated in 0.06 M Tris-HCl (pH 6.8) buffer without osmoticum (sample buffer). Subsequent solubilization of the chloroplast sample with SDS in a Chl:SDS ratio of 1:10, and separation of the complexes on 10% SDS-polyacrylamide gels were done according to the method of Krishnan and Gnanam (14).

Photosynthetic Measurements. O_2 exchange by the cultured cells was determined at 30°C following the method described earlier (6). Unless otherwise stated, the reaction mixture in a final volume of 1 ml contained 50 mM sodium phosphate (pH 7.2), 20 mM NaCl, 5 mM $MgCl_2$, 5 mM $NaHCO_3$, and cells corresponding to 15 to 20 μg Chl. PSII activity in isolated chloroplasts was estimated by ferricyanide and DCIP reduction assays using a Bausch and Lomb Spectronic 70 spectrophotometer. PSI was assayed polarographically with DCIP-ascorbate as the electron donor and MV as electron acceptor (16).

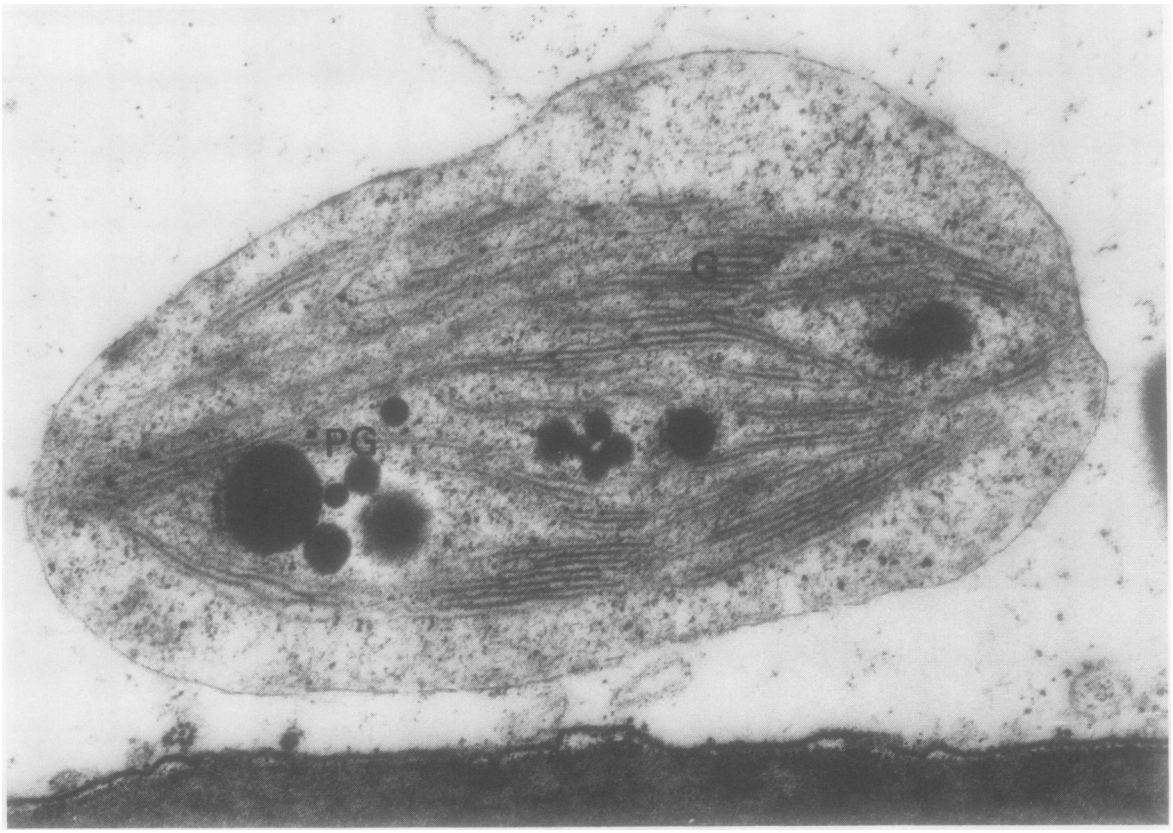

FIG. 3. Electron micrograph of a chloroplast of *A. hypogaea* var. TMV-3 cell showing well-differentiated grana (G). PG, plastoglobules (× 36,000).

Fluorescence Measurements. Induction of Chl fluorescence was followed in mesophyll and cultured cells after excitation with broad band blue light (400–460 nm) (Corning 5113) at an irradiance of 10 w m^{-2}. The photomultiplier (Hamamatzu R375) placed 90° to the excitation beam was protected by an interference filter (X max, 690 nm; half band width, 12 nm; Schott). The signal from the photomultiplier was directly displayed on a servo recorder. The cells at a final Chl concentration of 0.5 μg/ml were suspended in a buffer containing 50 mM sodium phosphate (pH 7.2), 20 mM NaCl, 5 mM $MgCl_2$, and 0.3 M sucrose. For DCMU treatment, the cells were vacuum infiltrated with the inhibitor (5 μM) in the dark at 25°C. Control cells were treated under identical conditions with the buffer.

Estimation of Pigments and Proteins. Chl/carotenoids of the cells and gel portions containing the Chl-protein complexes were extracted in 80% cold acetone. Chl content of the extracts was determined using the constants of Arnon (1). Carotenoids were estimated from the *A* at 473 nm using an extinction coefficient of $\frac{E\%}{1\ cm} = 2{,}500$.

Proteins in the 10% TCA extracts of the cells were determined by the method of Lowry *et al.* (16). Crystalline BSA served as the standard.

RESULTS

The cultured cells of *A. hypogaea* were found to occur in both free and aggregate (5–50 cells) forms. Although the cells were mostly free in the agitated medium, they tended to aggregate in still medium (Fig. 1A). The cell aggregates were, however, very loose as swirling of the culture flasks for a few seconds once in a day resulted in the complete separation and growth of free cells (Fig. 1B). Even otherwise, free cells could be easily obtained by passing the cell clusters through a nylon sieve (211 μm) with mild stirring. The cells thus obtained were spherical, ovoid, or elongated and contained a large number of well-differentiated chloroplasts (Fig. 2). The chloroplasts were characterized by well-developed grana (Fig. 3) and were similar to those of the mesophyll cells of *Arachis* leaves *in situ*. The chloroplasts contained prominent plastoglobules but lacked starch. The number of chloroplasts per cell varied from 50 to 105. The amount of Chl on a gram dry weight basis varied from 3.85 to 4.44 mg (210–240 μg/g fresh weight) which amounts to approximately 15% to 20% of the total Chl in the leaves.

When the cells were transferred to fresh medium, an increase in cell number and dry weight occurred, after a lag of 3 to 4 d. Substantial increase in the protein content of the cells however, was evident during this lag period (Fig. 4). The generation time of the cells during the period of rapid growth (5–25 d) was between 3 and 4 d. During the stationary phase after 35 d, a marginal decline in dry weight and protein content of the cells without being followed by the number of cells and PCV was observed. Though the pattern of growth of the cells in both agitated and still media was similar, the latter medium promoted marginally more synthesis of Chl and carotenoids. The pigment content increased with dry weight and cell number throughout the log phase. The Chl *a/b* ratio (2.6), however, remained practically constant during the entire span of growth of the cells.

The growth of the cell cultures derived from the friable calli of a few other plant species representing different photosynthetic options in agitated and still nutrient media was also studied (Table I). While in a few plants, the rates of growth and Chl synthesis of the cells were less in still media, the cell cultures of *Nicotiana* and *Kalanchoë* gradually lost Chl and showed signs of death as deduced from neutral red dye staining. The growth and pigment synthesis in cell suspensions of *Gisekia* in still medium were comparable to that of the shake cultures. However, the marginally higher Chl content of the still-grown cells of *Arachis* was not

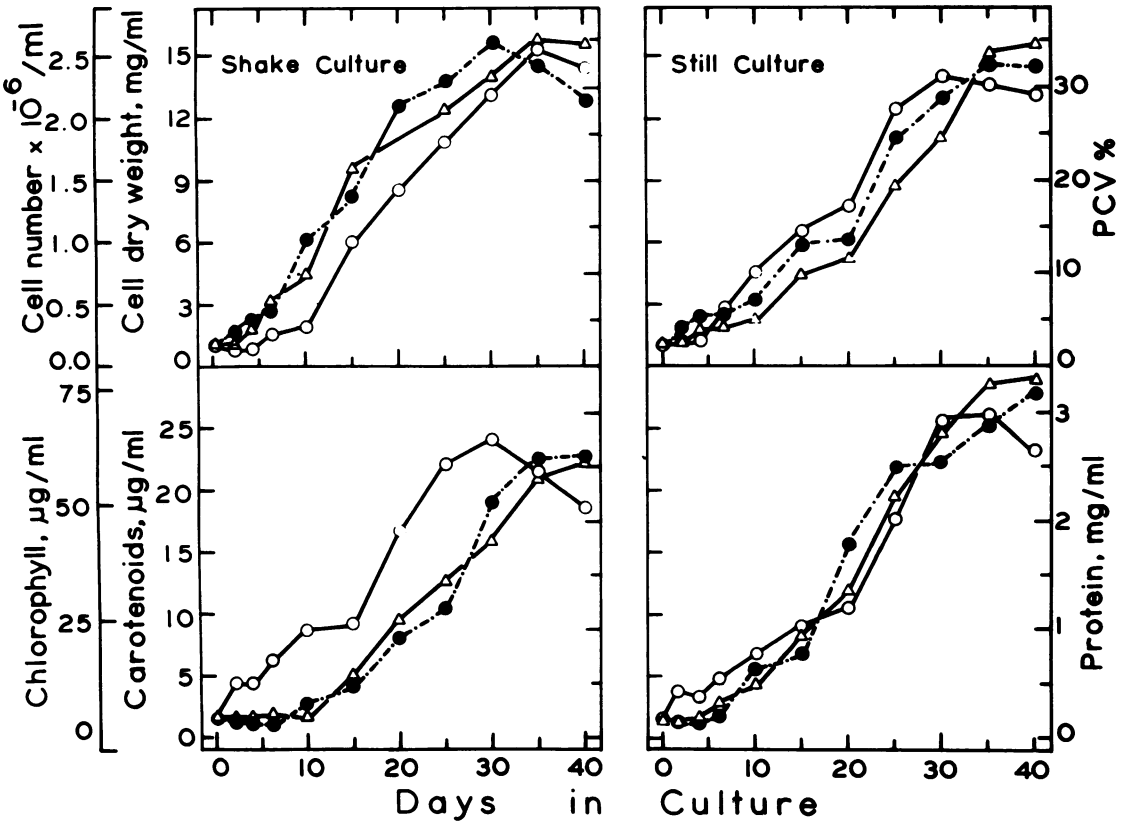

FIG. 4. Relative changes of growth, pigments, and protein in suspension-cultured cells of *A. hypogaea* var. TMV-3 in still and agitated media. PCV and protein (○); cell dry weight and carotenoids (●); cell number and Chl (△).

Table I. *Comparative Rates of Growth and Chl Synthesis in Photoheterotrophic Cell Suspension of Some C_3, C_4, and CAM Plants Grown in Still and Agitated Media*

The inoculum consisted of cells weighing approximately 1.5 g fresh wt (80 mg dry wt). Absolute values of Chl (µg/100 mg dry wt) for the inocula were: *A. hypogaea*, 413; *N. tabacum*, 223; *G. pharnaceoides*, 286; *P. oleracea*, 314; *C. umbellata*, 216; *C. sylvestrii*, 325; *K. verticillata*, 434. Observations were made after 3 weeks of growth. The values are the mean ±SD of four replicates of two different experiments.

Plant	Dry Wt		Chl	
	Still culture	Shake culture	Still culture	Shake culture
	% increase or decrease		*% increase or decrease*	
C_3				
Arachis hypogaea L.	905 ± 201	924 ± 109	952 ± 184	807 ± 92
Nicotiana tabacum L.	−58 ± 8	1054 ± 148	−20 ± 5	790 ± 21
C_4				
Gisekia pharnaceoides L.	1105 ± 117	1152 ± 214	464 ± 85	809 ± 123
Portulaca oleracea L.	579 ± 63	801 ± 134	475 ± 42	729 ± 81
CAM				
Caralluma umbellata Hav.	126 ± 23	522 ± 56	15 ± 3	348 ± 42
Chamaecereus sylvestrii Spegazzini	644 ± 117	983 ± 121	508 ± 64	908 ± 88
Kalanchoe verticillata Elliot	−48 ± 6	−632 ± 119	−72 ± 6	711 ± 26

observed in these cells.

When a number of varieties of peanut were screened for the establishment of chlorophyllous cell cultures in still medium, marked differences in growth, pigment content, friablity (yield of free cells), and photosynthetic O_2 evolution capacity were observed (Table II). Only three varieties, *viz.* TMV-1, TMV-3, and 117010 A NC 4-X Virginia peanuts were found to possess significant rates of growth (876–992% increase in fresh weight in 3 weeks), Chl synthesis (193–217 µg/g fresh weight), friability (78–85%), and photosynthetic activity (134–154 µmol O_2/mg Chl·h). TLC separation of the pigments of cell cultures of *A. hypogaea* var. TMV-3 showed all the principal pigments of the leaf tissue in the same ratio but at lower concentrations (Table III). However, the Chl to carotenoid ratio was somewhat lower than that of the intact leaves.

The endogenous rates of O_2 evolution by the cultured cells of *Arachis* were lower (10–20 µmol/mg Chl·h) and lasted for only 5 min. Addition of HCO_3^- stimulated O_2 evolution and maximal rates of O_2 evolution (140–170 µmol/mg Chl·h) occurred when 5

Table II. *Comparative Rates of Growth, Chl Synthesis, Friability of the Cell Aggregates, and Photosynthetic O_2 Evolution in Cell Cultures Established from Different Varieties of A. Hypogaea*

Calli initially proliferated upon the leaf explants and subsequently subcultured on MS basal agar medium supplemented with NAA, 0.6 mg/l; BA, 0.2 mg/l; and glucose, 3.5% were transferred to the liquid medium. Observations were made after 3 weeks. The results represent the mean of the values obtained from three consecutive passages, each consisting of at least two different samples.

Variety	Fresh Wt	Chl	Yield of Free Cells	O_2 Evolution	
	% increase	*μg/g fresh wt*	*%*	*μmol/mg Chl·h*	*μmol/g fresh wt*
TMV-1	934	202	78	134	27
TMV-2	647	131	71	97	13
TMV-3	992	217	85	154	33
TMV-7	684	120	72	106	13
TMV-9	676	86	58	84	7
TMV-10	592	132	69	132	17
M-13	876	141	81	142	20
Robut-33-1	635	125	72	103	13
Gangapuri	812	108	74	112	12
MH-2	784	119	76	99	12
J-11	737	149	69	129	19
Sm 5	817	151	83	118	18
NcACC 170090	789	162	65	101	16
EC 76446	856	118	79	98	12
EC 117010 A NC4-X Virginia	876	193	85	139	27
PI 259747	674	127	69	106	14

Table III. *Pigment Composition of the Intact Leaf Tissue and Photoheterotrophic Cells of A. hypogaea var. TMV-3 Grown in Still Nutrient Medium*

The pigments were extracted in 90% (v/v) methanol and subsequently separated by TLC. The details are given in "Materials and Methods." The values are the mean of two separate determinations.

Pigment	Leaf	Cell Culture
	μg/100 mg dry wt	
Chl *a*	1,346	274
Chl *b*	541	113
Total Chl	1,887	387
α + β Carotenes	138.4	30.4
Lutein	189.7	43.3
Lutein epoxide	38.7	7.2
Violoxanthin	29.4	6.3
Neoxanthin	66.0	15.6
Total carotenoids	462.2	102.8
Chl *a/b*	2.49	2.42
Chl/carotenoids	4.08	3.76

to 10 mM $NaHCO_3$ was included in the reaction medium (Fig. 5). The light-dependent O_2 evolution was sensitive to 5 μM DCMU. The chloroplasts isolated from the cell cultures mediated both PSI and PSII functions as well as photophosphorylations (Table IV). On a Chl basis, such functions were similar to or more than those of the plastids isolated from the leaves. The variable fluorescence (change of the initial fluorescence to the steady-state range) of the cultured cells was also comparable to that of the leaf cells (Fig. 6). Addition of 5 μM DCMU to the cells inhibited the variable fluorescence and yielded maximal fluorescence throughout the illumination period.

In chloroplasts of the mesophyll cells, the light-absorbing pigments occur as pigment-protein complexes. Since pigment-associated polypeptides constitute the structural basis for the photochemical events, various complexes in a relatively pure form from chloroplasts of the cultured cells using SDS-polyacrylamide gel electrophoresis were separated. The solubilized chloroplasts after short-term electrophoresis, separated into three major Chl-containing bands, corresponding to CPI, CPII, and free pigment zone in the order of increasing mobility. The relative distribution of the Chl pigments and the Chl *a/b* ratio in the Chl containing bands are shown in Table V. The CPI accounted for 20% to 30% of the total Chl loaded, while the CPII and free pigment zones accounted for 30% to 40% and 30% to 45% of the total, respectively. The CPI contained mostly Chl *a*, while the CPII contained both Chl *a* and Chl *b*. In chloroplasts isolated from the leaf tissues, however, the Chl concentrations of the CPI, CPII, and FP accounted for 10% to 20%, 40% to 50%, and 40% to 50% of the total, respectively.

DISCUSSION

Growth Characteristics. The sigmoidal curve obtained with the growth of the cultured cells of *Arachis* closely agrees with that of other chlorophyllous cell cultures already described (8, 18, 21). The growth of the cells in still media, however, is considered significant as it is confined to a few plant species.

The observed increase in Chl content of the peanut cells grown in still medium, perhaps, might be related to the gas phase developed over the cultures. Such cultures compared to the shake-grown ones might have lower than normal levels of O_2 and higher CO_2 besides any specific volatile substance in the gas phase developed over the cultures. This is in line with the observations of Dalton and Street (8) that lowered O_2 partial pressure and greater CO_2 accumulation in the gas phase is a primary cause of greening in cell cultures of spinach. The parallel increase in Chl concentration closely following that of growth during the exponential phase is consistent with the observations made in a few other culture systems (18, 21).

The pigment concentration of the cell cultures (210–240 μg Chl/g fresh weight) of *A. hypogaea* var. TMV-3 could be well compared with the values of Chl reported previously in callus and cell cultures. Even the highly chlorophyllous cultures reported thus far, contained only 120 to 180 μg Chl on a g fresh weight basis (13, 20). Another desirable feature is the highly dispersed status of these cells in the culture. Even the already present cell aggregates in still media readily yielded free cells on shaking the flasks for a

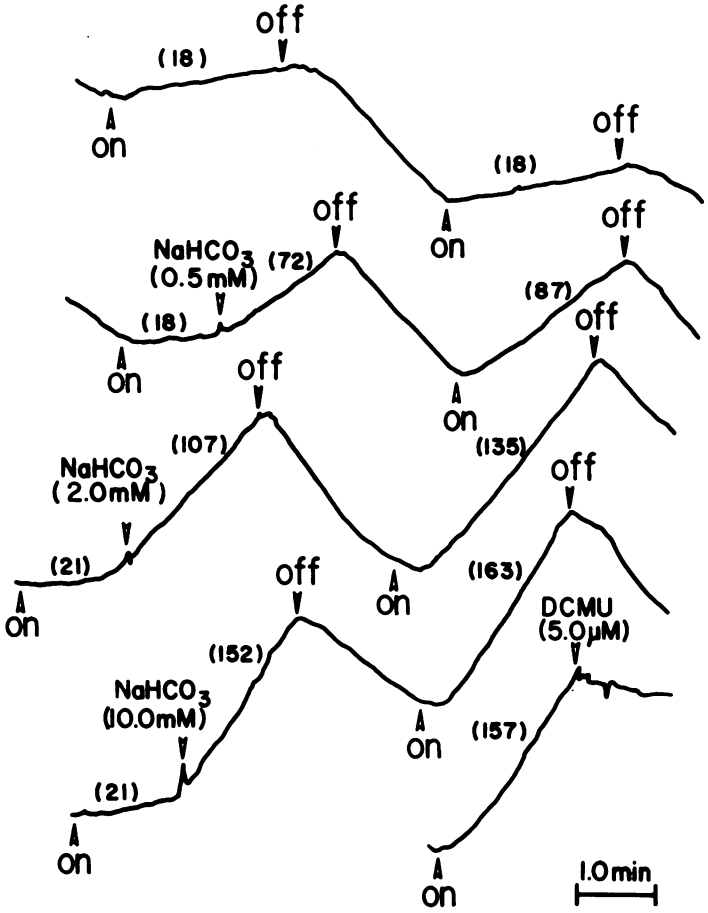

FIG. 5. Polarographic tracings of O_2 evolution by suspension-cultured cells of *A. hypogaea* var. TMV-3 in presence of varied concentrations of bicarbonate. Concentration of DCMU used was 5 μm. Figures in parentheses indicate rates of O_2 evolution in μmol/mg Chl·h.

Table IV. *Photochemical Activities of Chloroplasts Isolated from Intact Leaf Tissue and Photoheterotrophic Cells of A. hypogaea var. TMV-3 Grown in Still Nutrient Medium*

Ferricyanide reduction was determined as the light-induced decrease in 420 nm absorption; DCIP reduction was measured as the light-induced decrease in 610 nm absorption; MV stimulated O_2 uptake in the presence of DCMU, and ascorbate was determined polarographically. Other details are given elsewhere (6). The values are the mean of three separate determinations.

Reaction Measured	Chloroplasts from Cell Cultures	Chloroplasts from Leaf
	μmol/mg Chl·h	
Electron transport		
Ferricyanide reduction	124	99
DCIP reduction with DPC	114	92
MV reduction with DCIP-ascorbate couple	234	188
Photophosphorylation		
Noncyclic	112	137
Cyclic	251	209

few seconds or on being passed through a suitable sieve with mild stirring. The comprehensive information available in literature suggests that such cells are more often the exception than the rule and are paralleled only by the well-dispersed heterotrophic cell

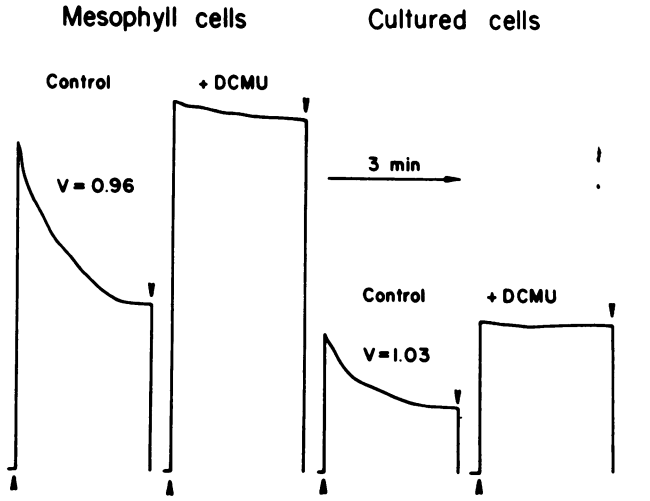

FIG. 6. Relative levels of fluorescence in mesophyll and suspension-cultured cells of *A. hypogaea* var. TMV-3. Cells suspended at a final Chl concentration of 0.5 μg/ml were used. Other conditions of measurements as in "Materials and Methods."

Table V. *Distribution of Chl in Chl-Protein Complexes of SDS-Solubilized Thylakoids of Chloroplasts Isolated from Leaves and Photoheterotrophic Cells of A. hypogaea* var. *TMV-3 Grown in Still Nutrient Medium.*

Chl-protein complexes separated on 10% SDS-polyacrylamide gels (Fig. 3) were extracted with 80% cold acetone, and total Chl and Chl *a/b* values were determined using the constants of Arnon (1).

Assay	CPI	CPII	FP
Chloroplasts isolated from the leaf			
% of Chl	15.0	43.0	42.0
Chl *a/b*	5.7	1.9	4.1
Chloroplasts isolated from cell cultures			
% of Chl	29.0	37.0	34.0
Chl *a/b*	5.3	1.4	3.3

cultures of *Acer pseudoplatanus* (23).

Photosynthetic Characteristics. The observed rates of O_2 evolution by the cell cultures of *Arachis* are significantly high compared to most of the callus and cell suspension cultures described by others (3, 5, 12, 18). It has been suggested that the light-dependent O_2 evolution without added HCO_3^- in leaf cells and cell cultures is due to the availability of endogenous CO_2 liberated by the decarboxylation of malate (21, 22). Accordingly, the failure of the cell cultures of *Arachis* to evolve O_2 endogenously might be related to the lack of endogenous CO_2 concentration. The relatively low labeling of malate during short-term $^{14}CO_2$ incorporation in the late log-phase cell cultures (results presented elsewhere) might indicate the very low endogenous concentration of malate to liberate CO_2 through the decarboxylation process. However, the observation that even the early log-phase (10-d-old) cells which predominantly synthesize malate in short-term CO_2 fixation do not exhibit substantial endogenous rate of O_2 evolution is surprising. Vanderhoven and Zyrd (25) proposed that in cell cultures the endogenous malate could be utilized either through the tricarboxylic acid cycle or through decarboxylation liberating CO_2. The less competent carboxylating system of even the log-phase cells to support O_2 evolution by releasing CO_2 through malate oxidation implies that the endogenous malate is mainly used in the respiratory cycle.

The presence of well-organized grana in the chloroplasts of the

cell cultures, DCMU-sensitive O_2 evolution, and presence of fully active PSI, PSII, and photophosphorylation components of the chloroplasts well document the development of functionally active photosynthetic apparatus in the cultured cells. This is strengthened by the pattern of variable fluorescence obtained with the cultured cells which is a direct indication of the existence of PSII activity and photolysis of water (4, 9, 15). The results are significant in view of the fact that studies on photochemical properties of the cultured cells are scarce (20).

The development of functional photosystem in the cell cultures of *Arachis* is further validated by the detection on SDS-polyacrylamide gel electrophoresis of chlorophyll-protein complexes associated with both PSI and PSII functions. The marginal decrease in the percent of Chl associated with CPII and the near doubling of the percent seen in CPI in the cultured cells as compared to intact leaves may have significant implications regarding the photosynthetic competence of cultured cells and remains to be investigated.

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